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On the experimental Studies and field Observations on the Relationships between the pH of the soils and the Pupulation density of the Citrus Nematode, Tylenchulus semi-penetrans

by

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Introduction

The citrus nematode, *Tylenchulus semi-penetrans*, was first discovered attacking citrus in California by J. R. Hodges in 1912. And this narasite has been reported in America, Argentin, Algiers, Australia, Brazil, Canary Island, Chili, India, Israel, Egypt, France, Italy, Japan, Malta, Natal, Pakistan, Parestine, Russia, Spain and South Africa etc., distributing in all the citrus growing areas of the world.

In Japan the citrus nematode was first found at Tokushima horticultural experimental station in 1957, and can be considered to widely distribute in the citrus growing regions in Japan. (18) But little is known about the relationship between the soil texture and the reproduction of this nematode in Japan.

Observations on this relationship in Argentina, Australia, South Africa, and California show that the citrus nematode is capable of living under a wide range of field conditions (1, 2, 3, 4). But more citrus nematodes, and other nematodes too, were found around citrus roots subjected to continuous high, though tolerable salinity, than around citrus roots grown at lowere salinity. (5) And in 1961 Van Gundy and Martin found that there are many factors affecting the population density of this nematode, but of all factors soil pH may be more important factor indicating that the soils with pH below 4.9 had lower populations over a period of 7 months croping to citrus seedlings than soils above pH 4.9 (6). And at acid reactions above pH 5.0 acidity may act as a limiting factor for the total population of this nematode; and the nematodes survived at pH 3.3, but reproduction ceased. (6) And in 1962 they also found that a neutral textured soil (pH 7.0) contained 4 times as many citrus nematode larvae as an acid loam (pH 5.0) or an acid neutral sand loam. (7) In 1963 they also found that the numbers of citrus nematode increased most rapidly in sandy loam, but reached maximum levels in an alkaline clay soil loam; and the number of this nematode could be lessened by the addition of the root phosphorous in excess of 0.3 % (8). In 1966 Kirkpatrick and Van Gundy found that disccusing from the relationship between soil salinity and citrus nematode survival, citrus nematode survived in soils of pF values 3.68 and 4.15 may be related, in

part, to a conservation of energy through a general reduction in nematode energy; and the mortality rate of the males, was essentially normal, l. e., increased after 8 days with virtually none surviving after 68 days. (9) On the other hand, the damages of roots of orange, madarin and lemon caused by the citrus nematodes are severe in sandy soil, (10) and are usually most severe under soil conditions that are borderline for the growth of citrus; and the nematode injury is accerated by low trace element availability and more exchangeable Na or K % in the soils. (11) In 1964 Van Gundy et al. found that the seedling growth reduction due to the citrus nematode was greater in wetter soils than in drier soils; and nematode reproduction was favoured by a dry conditions in fine drier soils. (12)

Considering from these reports, soil moisture and pH values of the soils are the limiting factors for the attack of the citrus nematode to their host plants, and the soil texture seemed to be the modifying factors for the reproduction of the citrus nematode, as stated by L. H. Stolz and S. D. Gundy. (12, 13) And the production and development of eggs of the citrus nematode may be reduced in soils with low oxygen availability, and if a soil is wet most of time, the reproductive processes are slowed down. Low oxygen levels which affects nematode also affect the growth of citrus plants and may contribute to the total growth reduction due to the citrus nematode. And the aeration, as related to moisture and clay content, is probably most improtant in the fine texture soils which dry out slowly. In the very sandy soils, pores of soil are either full of water or nearly empty and thus go from one adverse reproduction to, another with very little time in between to favor optimum reproduction of the citrus nematode. And the acid reaction above pH 5.0 of the soils may act as a limiting factor for the total population density of the citrus nematode.

On the other hand, the damages of the host plants caused by the citrus nematode are different according to the species of the host plant. In 1948 E. P. DuCharme (14) and R. C. Baines, O. F. Clarke and W. P. Bitters (15) found that all the common species of Citrus are highly susceptible to the citrus nematode, and but the trifoliate orange *Poncirus trifoliata* is markedly resistant, although not immune. In 1962 B. A. Oteifa and A. T. Sharawi (16) found that *T. semi-penetrans* appeared to be associated with poor growth of Citrus in more than half of the 743 cases tested and is probably a widespread and important parasite of this crop. 31 species and varieties of Citrus plants (*Citrus* and *Fortunella spp.*) were found infested, but no hosts could be found outside this group. Orange nursery stocks appeared to be generally infested.

The 9 citrus rootstocks available were all infested, but indications were obtained that *Citrus aurantinm var.* THORNESS and *C. aurantinium amara var.* SWEET were more resistant than the others. In 1954 J. W. Cameron; R. C. Baines and D. F. Clarke stated that common species of Citrus were susceptible to the citrus nematode, whereas the trifoliate orange, *Poncirus trifolitata*, is markedly resistant. Young seedling population of *Poncirus* from 3 source all showed a high degree of nematode resistance. Hybrids seedling populations were obtained from crosses between *Poncirus* and 5 *Citrus* species.

These populations likewise all showed marked resistance, whereas accompanying nuclar seedlings of Citrus were usually susceptible. Orchard trees of several older hybrids between *Poncirus* and *Citrus* have shown moderate or severe iufestation by the nematode. This may reflect differences in genetic composition or a lesisening of resistance in older

trees. (17)

In 1963 S. D. Van Gundy and J. P. Kirkpatrick reported the results of investigations on the histological relationship of resistance to the citrus nematode in certain Citrus rootstocks, showed thate seedlings of *Severinia buxifolia* (immune), *Poncirus trifoliata* (resist ant) and other *Citrus jabhir* (susecptible) were infested with second stage larvae of the citrusnematode. In the susceptible host the cells on which the nematodes were feeding filled with protoplasma and the nuclars enlarged. In the resistant and the immune hosts entry and feeding of the 2nd stage larvae resulted in the deposition of suberin between the cells of the hypodermis and of the cortex and a wound periderm was formed. Adult females were observed on susceptible roots at 4 weeks and first generation larvae at 9 weeks, but no females were found on the roots of the resistant and immune seedlings. (19)

In 1964 J. P. Kirpatrick and S. D. Van Gundy stated that the nature of resistance of *Poncirus trifoliata* as compared with *Citrus sinensis* and their hybrids appears to be associated with a hypersensitive cell reaction to feeding, a woundperiderm formation in the cortex, and the relative toxicity of the cell sap of the root. The feeding of the larvae stages of the citrus nematode caused the wound periderm formation in the seedlings of *Poncirus trifoliata* and the hybrids, but not in *C. sinensis*. A necrotic reaction of cells and suberization of cell walls around the heads of the feeding nematodes inhibited their develoment beyond the larvae stages in *P. trifoliata*. This reaction was not as severe in the hybrids which allowed a few larvae to develop into young females. The relative toxicity of expressed sap of root to the second stage laevae was greater in *P. trifoliata* than in either *C. sinensis* or the hybrids. The lack of any quantitative difference in the toxicity of root sap and the reduced intensity of the wound reaction in the hybrids may account for the eventual development of a certain nematode population to the some extent on a hybrid root as on a *C. sinensis* root. (20)

In Japan the regions at where the yearly mean air temperature is 15–16°C are considered to be the orange-belt, and the western regions of Japan are considered to be the promisible citrus growing district. And in these regions the mountainous inclined slopes are mainely utilized for the main orange growing field. But the soils of the agricultural field in Japan are usually acidic textured, and the soil acidity is generally high in these mountainous regions, and these acidic soils are composed of heavy clay. And as the rootstocks of the orange seedlings the trifoliate orange (*Poncirus trifoliata*) are usually utilized.

Considering from these status of growing conditions of the citrus trees in the western districts of Japan, the reproduction of the citrus nematode in the field and the damages caused by this nematode can be considered to be not so severe as that in the another foreign countries where the soils are alkaline. But as the improvement of the agricultural construction progress, even the paddy fields near the foot of the mountain at where the soils are comparativelly weak acidic are recently transfered to the profitable citrus growing orchard in the orange belt of the western Japan. Disccusing from these recent status of the growing of the citrus tree, the reproduction of the citrus nematode in the orchard soils and the damage caused by this neamatode will be promoted year by year in future.

From these standpoint of views referring to the status of the citrus nematode in the western district of Japan, we investigated the relationship between the pH of the soils of orange orchards and the population density of the citrus nematode, and experienced the

population density of the citrus nematode in the soils of which the pH are controlled by artificial methods to pH 4.5, 6.6 and 7.7.

Experiment I

Materials and Methods

We prepared the experimental steam-sterilized soils with known pH value in which we added the soils, which the density of population of the citrus nematode are previously investigated, to the above mentioned bed soils. And after about 50 days we investigated the numbers of the survived citrus nematode by Baermann's method. The pH values of the bed soil were contolled by the followin methods :

(1) Alkaline soil : pH 7.5－8.0 : 5 pots
The pH 9.0 Buffer solusion composed of Boron, Potassium chlorid, and Sodium hydroxide was poured into the sandy loam (pH 6.5)

(2) Neutral soil : pH 6.5－6.8 : 5 pots
The above mentioned sandy loam

(3) Acidic soil : pH 4.4－4.6 : 5 pots
The pH 3.4 Buffer solusoin composed of 1/5 M. CH_3COOH, 1/5 M. CH_3COONa and H_2O was poured to the brown sandy loam (pH 5.7)

These previously prepared neutral, alkaline, and acidic soils were packed into the 5 pots respectively. The diameter of each pot was 7 inches, and volume was about 4000 cm^3. And the soil containing citrus nematodes originated from Saga prefectural horticultural experimental satation (Ogi-Machi, Saga Prefecturs) (mean about 530 speciemens of larvae of the citrus nematode per 50 gr. soil) were added 100 gr. to each pot and mixtured with bed soil (inoculated-soil). These inoculated pots with citrus nematode were protected in the glass house. And after about 50 days 50 gr. soil from each pot was selected and investigated the number of the citrus nematode larvae survived by the Baermann's method.

The numbers of the citrus nematode larvae in the added soil originated from the horticultural experimental station were as follows :

Table 1 :

No. Sample	Citrus nematode Larvae/50 gr. soil	Nr. of another nematodes except Citrus nematode			Total
		Plant-par.	Free-liv.	Predac.	
1	546	73	216	17	852
2	420	24	210	20	674
3	525	44	310	35	914
4	531	8	263	21	823
5	613	24	315	28	980
Mean	527	35	263	24	849
%	62.0	4.3	31.0	2.7	100.0

The soils were collected at the end of August, 1965 showed pH. 5.5

Results

Table 2 :

Pot No.	pH (Starting)	Date of Starting	Date of Isolation of nemas	pH (Ending)	Nr. of Citrus nema (Larvae) **** 1	2	3	Total
Alkaline Soil :								
1	7.8	S. 40. 10. 16	S. 40. 12. 6	7.1	0	0	0	0
2	7.7	〃	4	7.1	0	0	0	0
3	7.5	〃	7	7.0	0	0	0	0
4	7.5	〃	5	6.9	0	0	0	0
5	8.0	〃	3	6.8	0	0	0	0
Mean	7.7	—	—	7.0	0	0	0	(0)
Neutral Soil :								
1	6.7	S. 40. 10. 16	S. 40. 12. 4	6.7	1	2	4	7
2	6.8	〃	5	6.5	0	1	0	1
3	6.5	〃	6	6.5	6	7	5	18
4	6.4	〃	7	6.5	4	1	1	6
5	6.5	〃	3	6.2	10	10	11	31
Mean	6.6	—	—	6.5	4.2	4.2	4.2	(4.2)
Acidic Soil :								
1	4.5	S. 40. 10. 16	S. 40. 12. 6	5.6	0	0	0	0
2	4.5	〃	7	5.6	0	2	0	2
3	4.4	〃	3	5.6	8	8	22	38
4	4.4	〃	5	5.7	0	1	0	1
5	4.6	〃	4	5.8	1	0	2	3
Mean	4.5	—	—	5.7	1.8	2.2	4.8	(2.9)

**** No. of Soil sample investigated

Disccusing from the above mentioned data, the number of the survived larvae of the Citrus nematode per 50 gr. soil were most numerous in the pot in which the soil was neutral, and was about 45 % of numbers in neutral soils in the acidic soil pot. And in the alkaline soil pot the numbers of the detected nematodes except the citrus nematode was very small (mean 10/50 gr. soil) in contrast to that in the acidic soil (mean 149) or the neutral soil (mean 234).

Comparing the numbers of the larvae of Citrus nematode in the soil at the starting time (mean 527/50 gr. soil) with that of the survived larvae after about 50 days, the mortality of the larvae was very great. But the fact that the total numbers of nematodes detected was also very small, amounting to 1.2 % (in alkaline soil), 28 % (in neutral soil) and 18 % (in acidic soil) respectivelly, seems to show the toxicity of the buffer solusions added to the soil for the controlling of pH to all nematodes.

Experiments 2

We investigated the relationships between the actual numbers of the larvae of Citrus nematode detected from the soils and the pH values of soils in the orange orchards.

Materials and Methods

The soils were collected from the area extending 40－150 cm horizontally and 10－15 cm in depth from the orange trees planted in the Saga prefectural horticltural experimental Station and in the orange growing region of Yamatocho, Saga-Gun, Saga Prefecture. And the nematodes were isolated from the soils by the Baermann's method.

Results

The results obtained in this experiment was as follows:

Table 3: Horticultural experimental Station (clayly loam soil)

No. of Samples	Date of Collection	pH of Soils	Nr. of larvae Citrus nema	Nr. of another nema	Total per5 0gr
1	11. 23. 1965	4. 47	34	52	95
2	〃	4. 68	31	55	86
3	〃	4. 70	57	111	168
4	〃	4. 75	76	129	505
5	〃	4. 80	108	197	305
6	〃	4. 83	134	258	392
7	〃	4. 90	326	530	856
8	〃	5. 05	568	1201	1769
9	〃	5. 40	1395	1438	2933
10	〃	5. 43	1501	1569	3070

Table 4: Yamatocho, Saga-Gun (Daiganji) (Black loam soil)

1	15. 12. 1965	5. 97	513	157	670
2	〃	6. 02	194	35	229
3	〃	6. 28	75	46	121
4	〃	6. 95	65	110	175
5	〃	7. 10	495	49	544

As shown in Table 3 and 4, the pH values of soil seemed to be correlated with the detected numbers of the Citrus nematode larvae generally.

Experiment 3

We experimented the resistance of the Trifoliate orange (*Poncirus trifoliata*) to the attack of the Citrus nematode comparing with that of the sour summer orange.

Materials and Methods

We prepared two series of the pot (Diameter 7 inches, unglazed). In one series of pot we packed the citrus nematode-containing soils originated from the Saga prefecrural horticural experimental Station, in which the larvae of the Citrus nematode were contained about 630 per 50 gr. of soil. And in another series of pot we packed the same soils sterilized by steam as above mentioned soil originated from the Station. And the nurseries of the Trifoliate orange and the sour summer orange were planted in these pots on 8 th, November, 1965 (after about 65 days), and we observed the growth of these nurseries and counted the numbers of female on the surface of the roots on 11 th, January, 1966.

Results

Table 5 :

	*	Pot No.	Nr. of the leayes Transplanting after 65 days (A)	(B)	Height growth (A) cm	(B)	Nr. of Female
Trifoliate Orange	S	1	36	31	14.0	14.8	0
		2	28	27	13.4	13.8	0
		3	23	23	14.6	15.2	0
		Mean	29.0	27.0	14.0	14.6	0
	N	1	26	15	12.4	12.6	1
		2	25	26	11.8	12.0	0
		3	24	20	10.0	11.0	1
		Mean	25.0	20.3	11.4	11.9	0.7
Sour Summer Orange	S	1	7	7	17.8	18.4	0
		2	8	9	15.8	16.2	0
		3	8	8	18.0	18.0	0
		Mean	7.7	8.0	17.2	17.5	0
	N	1	7	7	15.8	16.0	3
		2	13	14	15.4	15.8	5
		3	6	6	14.6	15.0	6
		Mean	8.7	9.0	15.3	15.6	4.6

* S ... sterilized Soil; N ... Nema-containing-Soil

Considering from the data as shown in Table 5, the damage of the transplanting could be observed in the pots of Trifoliate orange, but height growth of nurseries increased more or less after the transplanting. Female of the Citrus nematode could not be observed on the surface of the roots in the steam-sterilized soil in both nurseries, but were detected from the roots in the larvae containing soil. The density of female on the root of the summer orange was higher about 7 times than that of the trifoliate orange.

Summary

In Japan the citrus nematode distributes widely in the citrus growing regions, but the

damages caused by this nematode are not so severe and increases not so rapidly, that the growers of the citrus tree are generally unmidfull of the nematode in spite of the great density of population in the orchard soils.

There are many factors affecting to the reproduction and the damages of citrus trees caused by this nematode. And many reports hitherto issued concerning with this nematode in another foreign countries show that the alkalic loam wet soil seems to be favorable to the reproduction of this nematode in the soil, and the trifoliate orange seems to be restisant species to the parasitism of this nematode.

In Japan the citrus trees are generally planted in the clayly acidic loam soil on the declined dry mountainous fields, and the trifoliate orange are used to be utilized as the rootstock of the sweet orange.

Considering from these conditions of the citrus growing in Japan, we can suspect the status of the indifference on the cirrus nematode, but the density of this nematode in the soils increases year by year practually,

From these standpoiants of views we experimented the reactions of this nematode to the pH of the inhibiting soil and the parasitism to the rootstock, Triforiate orange.

Disccusing from the results obtained in some our experiments, the population density of the citrus nematode in the soil seemed to be evently correlated with the pH of the soil, and the resistance of the rootstock of Trifoliate orange to this nematode could be recognized.

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